specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Specifically, the Office Action stated that the specification lacked support for a cell that "does not contain a heterologous antibiotic resistance gene." Applicants have submitted a response on May 11, 2003, traversing the rejection. However, in the Advisory Action dated May 29, 2003, the rejection was maintained on the basis that the specification teaches the use of a pBR322 plasmid (which carriers two heterologous antibiotic resistance genes, Ampr and Tetr) to introduce the tsA58 gene into a cell, and that "Applicant does not teach that said antibiotic resistance genes should be removed; therefore, the skilled artisan would assume that the heterologous antibiotic resistance genes were still in the plasmid when the construct was introduced into the cells, and would be present in the resultant cells. . . ." Thus, the Examiner concludes that "[t]he use of antibiotic resistance genes is routine in the art and the skilled artisan would not understand based on the teachings of the specification that the claimed cells should not comprise such a gene." Applicants respectfully traverse this rejection.

Contrary to the Examiner's assertion, **the specification does it fact teach removal of the antibiotic resistance genes**. Applicants note the Examiner's reference to pages 13 and 14 of the specification, which teaches introduction of a large T-antigen gene into rat cells using a portion of pBR322. However, as described in detail on page 13, line 17, through page 14, line 6, of the specification, and in Ohno T. et al., Cytotechnology (1991) 7:165-172, cited therein, **the pBR322 vector, including the two antibiotic resistance genes, are spliced out of the DNA fragment T-antigen tsA58 prior to insertion into rat cells.** As discussed on pages 13 and 14 of the specification, the entire genome DNA of tsA58 without the SV40 replication origin is cloned into the BamHI site of vector pBR322 to make plasmid pSVtsA58ori(-)-2, which is then transfected into *E. Coli* to make large amount of plasmid DNA. The DNA fragment T-antigen tsA58 genomic sequences (about 5420bp) is then excised and separated from the plasmid DNA by BamHI enzyme digestion and gel purification. As will be appreciated by one of skill in the art, the resultant DNA fragment does not contain any vector sequence as the DNA fragment containing the tsA58 genomic sequences is cloned into and cut off the pBR322 vector via the same BamHI sites. Since the antibiotic resistance genes are on the pBR322

vector, the purified tsA58 genome sequences contains no vector sequences, including the two antibiotic resistance genes. Thus, because the purified DNA fragment (without pBR322 sequences) is introduced into the animal cells, the cells do not contain any heterologous antibiotic genes. (See line 11, page 18, through line 13, page 19, of the specification, and T. Ohno et al., *Cytotechnology*, (1991) 7:165-172, cited therein.)

Moreover, antibiotic resistance genes are not required in establishing the claimed cell lines of the present invention. As stated at page 3, lines 12-16 of the specification, primary culture cells collected from living tissues initially proliferate well, but the proliferation gradually declined and the cells die out. As is well known in the art, T-antigen of SV40 can immortalize cells. The present specification discloses the use of T-antigen tsA58, a temperature-sensitive mutant, to established immortalized cell lines. As stated in the specification, the purified DNA fragment containing the T-antigen tsA58 is microjected into male pronucleus of the pronucleus ova of a rat. The ova is then transplanted into the oviduct of a forest-mother (see page 14, lines 19-26, of the specification). The transfected T-antigen tsA58 gene is expressed in all somatic cells of the rat (see page 14, lines 2-6). After the transfection, cells are separated from tissues and incubated at permissive temperature (for example 33°C). Because tsA58 is actively expressed at permissive temperature, cells containing the transfected immortalizing gene tsA58 will divide at permissive temperature to form colonies, whereas untransfected cells will gradually show a reduced division rate and eventually die out (cellular senescence). Accordingly, through many generations and multiple rounds of subculture, one single immortalized cell line could be selected and isolated. (See Example 2 of the specification.)

Thus, Applicants respectfully submit that the specification has clearly conveyed to one skilled in the art that the heterologous antibiotic resistance genes are not required for selection and cloning of immortalized cells. In fact, the specification specifically teaches the removal of plasmid DNA containing antibiotic resistance genes.

Thus, in view of the above remarks and the previously filed Amendments dated May 11, 2003, Applicants submit that all issues relevant to patentability raised during the

prosecution of this application have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that the present application is in condition for allowance. Prompt and favorable consideration of this response is respectfully requested.

Respectfully submitted,

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